



From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
ETATS-UNIS D'AMERIQUE
in its capacity as elected Office

Date of mailing (day/month/year) 27 June 2001 (27.06.01)	
International application No. PCT/CA00/01162	Applicant's or agent's file reference 10447-14
International filing date (day/month/year) 04 October 2000 (04.10.00)	Priority date (day/month/year) 04 October 1999 (04.10.99)
Applicant BRAUN, Curtis et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:
03 May 2001 (03.05.01)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was
☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Odile ALIU Telephone No.: (41-22) 338.83.38
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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 10447-14	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/CA 00/ 01162	International filing date (day/month/year) 04/10/2000	(Earliest) Priority Date (day/month/year) 04/10/1999
Applicant TWINSTRAND THERAPEUTICS INC.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1- 42 all partially

Nucleic acid molecule encoding a protein comprising an A chain of a ricin-like toxin, a B-chain of a ricin-like toxin linked together by a linker sequence containing a cleavage recognition site for a specific protease which is MMP-9.

2. Claims: 1-42 all partially

Nucleic acid molecule encoding a protein comprising an A chain of a ricin-like toxin, a B-chain of a ricin-like toxin linked together by a linker sequence containing a cleavage recognition site for a specific protease which is UPA.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 00/01162

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 22-30 (as far as they relate to an in vivo method) are directed to a method of treatment of the human/animal body (rule 39.1 IV PCT), the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 00/01162

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Although claims 22-30 (as far as they relate to an in vivo method) are directed to a method of treatment of the human/animal body (rule 39.1 IV PCT), the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1- 42 all partially

Nucleic acid molecule encoding a protein comprising an A chain of a ricin-like toxin, a B-chain of a ricin-like toxin linked together by a linker sequence containing a cleavage recognition site for a specific protease which is MMP-9.

2. Claims: 1-42 all partially

Nucleic acid molecule encoding a protein comprising an A chain of a ricin-like toxin, a B-chain of a ricin-like toxin linked together by a linker sequence containing a cleavage recognition site for a specific protease which is UPA.

INTERNATIONAL SEARCH REPORT

International Application No.

CA 00/01162

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/29 C12N15/62 C07K14/415 A61K38/16 A61K31/70
C07K14/00 A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, SEQUENCE SEARCH, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 49311 A (NOVO ENZYME CORP DE ;BORGFORDE THOR (CA)) 5 November 1998 (1998-11-05) cited in the application figures 5A,B,C,D figures 39,A-D page 14, line 3 -page 17 page 9, line 15 - line 18 page 12, line 6	1-8, 11-20, 22-40
A	claims; examples 4-6,8	9,10,21, 41,42
A	WO 89 04839 A (GENETICS INST) 1 June 1989 (1989-06-01) sequence ID no.1	1-42

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

6 November 2001

Date of mailing of the international search report

16.11.01

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
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Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
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Authorized officer

Le Cornec, N

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

CA 00/01162

Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 9849311	A	05-11-1998	AU	7023798 A	24-11-1998
			WO	9849311 A2	05-11-1998
			EP	0977862 A2	09-02-2000
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WO 8904839	A	01-06-1989	EP	0341304 A1	15-11-1989
			JP	2502287 T	26-07-1990
			WO	8904839 A1	01-06-1989
<hr/>					

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 April 2001 (12.04.2001)

PCT

(10) International Publication Number
WO 01/25267 A2

- (51) International Patent Classification⁷: **C07K 14/00**
- (21) International Application Number: **PCT/CA00/01162**
- (22) International Filing Date: 4 October 2000 (04.10.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data: **64 Apr-02/30 ms**
60/157,807 4 October 1999 (04.10.1999) US
60/197,409 14 April 2000 (14.04.2000) US
- (71) Applicant (for all designated States except US): **TWIN-STRAND THERAPEUTICS INC.** [CA/CA]; 8081 Lougheed Highway, Burnaby, British Columbia V5A 1W9 (CA).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **BRAUN, Curtis** [CA/CA]; 11519 140A Street, Surrey, British Columbia V3R 3J1 (CA). **PURAC, Admir** [CA/CA]; Apt. 2001, 3980 Carrigan Court, Burnaby, British Columbia V3N 4S6 (CA). **BORGFORD, Thor** [CA/CA]; Apt. 206, 1690 Augusta Avenue, Burnby, British Columbia V5Z 2V6 (CA).
- (74) Agent: **BERESKIN & PARR**; 40 King Street West, 40th Floor, Toronto, Ontario M5H 3Y2 (CA).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— Without international search report and to be republished upon receipt of that report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **IMPROVED RICIN-LIKE TOXINS FOR TREATMENT OF CANCER**

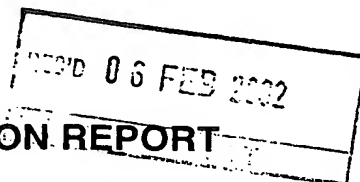
(57) Abstract: The present invention provides a protein having chain of a ricin-like toxin, a B chain of a ricin-like toxin and a novel heterologous linker amino acid sequence, linking the A and B chains. The linker sequence contains a cleavage recognition site for a specific protease such as those found in inflammatory cells and cancer cells. The invention also relates to a nucleic acid molecule encoding the protein and to expression vectors incorporating the nucleic acid molecule. Also provided is a method of inhibiting or destroying cells having a specific protease, such as cancer cells or inflammatory cells utilizing the nucleic acid molecules and proteins of the invention and pharmaceutical compositions for treating human inflammation and cancer.

WO 01/25267 A2

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)



Applicant's or agent's file reference 10447-14	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/CA00/01162	International filing date (day/month/year) 04/10/2000	Priority date (day/month/year) 04/10/1999
International Patent Classification (IPC) or national classification and IPC C07K14/00		
Applicant TWINSTRAND THERAPEUTICS INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.



2. This REPORT consists of a total of 7 sheets, including this cover sheet.

- ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand 03/05/2001	Date of completion of this report 04.02.2002
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Bretherick, J Telephone No. +49 89 2399 8415 

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/CA00/01162

I. Basis of the report

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-44 as originally filed

Claims, No.:

1-42 as originally filed

Drawings, sheets:

1/94-94/94 as originally filed

Sequence listing part of the description, pages:

1-27, filed with the letter of 13/12/2000

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/CA00/01162

- ☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:
5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):
(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:
- ☐ restricted the claims.
☒ paid additional fees.
☐ paid additional fees under protest.
☐ neither restricted nor paid additional fees.
2. ☐ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
- ☐ complied with.
☐ not complied with for the following reasons:
4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:
- ☒ all parts.
☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 9, 10, 21, 41, 42
 No: Claims 1-8, 11-20, 22-39

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/CA00/01162

Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-42
Industrial applicability (IA)	Yes:	Claims	1-21, 31-42
	No:	Claims	22-30, opinion reserved.

2. Citations and explanations
see separate sheet

R regarding Part IV (non-unity, R 13.1 PCT):

1. The International Examining Authority agrees with the opinion of the International Search Authority on the lack of Unity under R. 13.1 PCT of the current application.

2. There are 2 inventions as specified by the claims:

A: Claims 1-42 all partially,

Nucleic acid molecule encoding a protein comprising an A chain of a ricin-like toxin, a B-chain of a ricin-like cleavage recognition site for a specific protease which is MMP-9.

B: Claims 1-42, all partially.

Nucleic acid molecule encoding a protein comprising an A chain of a ricin-like toxin, a B-chain of a ricin-like cleavage recognition site for a specific protease which is UPA.

3. The provision of Ricin-like toxins by linking A-chain and B-chain of ricin by an heterologous linker specifically cleavable by a specific disease protease and specifically MMP-9 has been disclosed in the prior art. WO9849311 provides ricin-like toxin variants by linking the A-chain and the B-chain of ricin with a heterologous linker containing a cleavable recognition site for a disease specific protease for example with a linker recognised by MMP-9 (fig. 5A-D) for the treatment of cancer, viral or parasitic infections.

In view of this prior art, the problem underlying the application can be defined as the provision of further linkers to link chain-A and Chain-B of ricin.

The solutions proposed in the underlying application are summarised as follows:

The provision of linker sequences containing a cleavage recognition site for the specific protease MMP-9.

The provision of linker sequences containing a cleavage recognition site for the specific protease UPA.

MMP-9 has been used in this context in the art and there is an essential structural difference existing between the two solutions. There are also no other common novel, special technical features discernable between the two invention groups. Consequently there is lack of unity within the meaning of R.13.1 PCT. The application can be considered to comprise two groups of inventions as communicated under Art. 17(3)(a) PCT.

4. Since examination fees have been paid for both invention groups, all claimed subject-matter has been examined.

Regarding Part V: (Art. 33 PCT):

1. WO 98 49311 is a disclosure of modified recombinant ricin toxins where ricin chains A and B are separated by an heterologous linker which is cleavable by proteases which are specific to a particular pathogenicity, for example breast or ovarian cancer, (for example, MMP-9 figures 1-7, esp. 5A-D, 11-). Other linkers are cleavable by the protease UPA, (for example, figures 8-10).

Medical formulations containing same as well as methods of treatment are also disclosed therein.

The subject-matter of claims 1-8, 11-20 and 22-40 is thereby not new (Art. 33(1)(2) PCT).

2. The remaining claimed subject-matter, being variants on the above is not considered to involve an inventive step under Art. 33(1)(3) PCT.

Claim 9 recites the use of the linker which is encoded by construct pAP302 (SEQ ID NO:11). This bears significant structural similarity to a part of the linker in pap220. (100% overlap over 50 nucleotides). It is considered that, bearing in mind the numbers of variant solutions provided in the above cited art, that the claimed

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/CA00/01162

subject-matter represents yet more variant solutions to the known general technical problem of the provision of alternative linkers or alternative toxin conjugates in this context. The skilled person would appreciate that further variants would be possible with a high degree of certainty. The current variants have not been described as having any great advantages, nor are surprising effects associated therewith. An inventive step is therefore not accorded thereto.

3. Claims 22-30 are directed to methods of treatment of the human or animal body. An opinion regarding industrial activity Art . 33(1)(4) PCT is reserved, since there are no common, unified criteria within the PCT for the establishment of such an assessment of claims of this type.